

CLAIMS

1. A method of diagnosing *Mycobacterium tuberculosis* infection in a human, or
5 of determining whether a human has been exposed to *Mycobacterium tuberculosis*, comprising:
 - (i) contacting T-cells from said human with one or more of
 - (a) a peptide having the sequence shown in SEQ ID NO: 1;
 - (b) a peptide having or comprising the sequence of at least 8 consecutive
amino acids of the sequence shown in SEQ ID NO: 1; or
 - 10 (c) a peptide having or comprising a sequence which is capable of
binding to a T-cell receptor which recognises a peptide as defined in (a) or (b); and
 - (ii) determining whether any of the said T-cells recognise said peptide,
wherein steps (i) and (ii) are optionally carried out *in vitro*.
- 15 2. A method of increasing the sensitivity of a diagnostic test for diagnosing
Mycobacterium tuberculosis infection in a human, wherein said diagnostic test comprises
contacting T cells from said human with a *Mycobacterium tuberculosis* antigen which is not
Rv3879c, said method additionally comprising
 - (i) contacting T-cells from said human with one or more of
 - 20 (a) a peptide having the sequence shown in SEQ ID NO: 1;
 - (b) a peptide having or comprising the sequence of at least 8 consecutive
amino acids of the sequence shown in SEQ ID NO: 1; or
 - (c) a peptide having or comprising a sequence which is capable of
binding to a T-cell receptor which recognises a peptide as defined in (a) or (b); and
 - 25 (ii) determining whether any of the said T-cells recognise said peptide,
wherein steps (i) and (ii) are optionally carried out *in vitro*.
3. A method according to claim 1 or 2, wherein step (i) further comprises
contacting said T-cells with one or more further *Mycobacterium tuberculosis* T-cell antigen(s) or
30 with an analogue(s) of said antigen(s) which is capable of binding to a T-cell receptor which
recognises said antigen(s).
4. A method according to claim 3, wherein said one or more further T-cell

antigens include antigens encoded by the RD-1 or RD-2 region, which antigens are preferably ESAT-6 and/or CFP10; or fragments thereof which are at least 8 amino acids long.

5. A method according to any one of claims 2 to 4, wherein said one or more
5 further T-cell antigens include Rv3873, Rv3878 or Rv1989c; or fragments thereof which are at least 8 amino acids long.
6. A method according to any one of the preceding claims, wherein step (i)
comprises contacting said sample of T-cells with two or more different peptides, each having the
10 sequence of at least 8 consecutive amino acids of the sequence shown in SEQ ID NO: 1.
7. A method according to any one of the preceding claims wherein peptides from,
or analogues of, at least five different antigens are contacted with the T cells.
- 15 8. A method according to any one of the preceding claims wherein one or more of the peptides
(i) represented by SEQ ID NO's 2 to 18, or
(ii) which bind to a T-cell which recognise (i), are contacted with the T cells.
- 20 9. A method according to any one of the preceding claims, wherein recognition of said peptide by said T-cells is determined by detecting the secretion of a cytokine from the T-cells.
10. A method according to claim 9, wherein the cytokine is IFN- γ .
- 25 11. A method according to claim 9 or 10, wherein said cytokine is detected by allowing said cytokine to bind to an immobilised antibody specific to said cytokine and detecting the presence of the antibody/cytokine complex.
- 30 12. A method according to any one of the preceding claims, wherein said T-cells are freshly isolated *ex vivo* cells.

13. A method according to any one of claims 1 to 11, wherein said T-cells have been cultured *in vitro*.

14. Use of

- 5 (i) a peptide as defined in claim 1 or 8, and optionally also an antigen as defined in any one of claims 3 to 5, or
- (ii) a polynucleotide which is capable of expressing (i),
in the manufacture of a diagnostic means for diagnosing *Mycobacterium tuberculosis* infection or exposure in a human.

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15. A diagnostic composition comprising a peptide as defined in claim 1 or 8 and optionally one or more further *Mycobacterium tuberculosis* T-cell antigens.

16. A composition according to claim 15 wherein said one or more further T-cell
15 antigens are selected from

- (i) ESAT-6, CFP10, Rv3873, Rv3878, Rv1989c or fragment of any thereof
which is at least 8 amino acids long; or
- (ii) an analogue of (i) which binds to a T-cell which recognises (i).

20 17. A kit for diagnosing *Mycobacterium tuberculosis* infection or exposure in a human, comprising one or more peptides as defined in claim 1 or 8 or a composition according to claim 15 or 16, and optionally a means for detecting recognition of a peptide by T-cells.

18. A kit according to claim 17, wherein said means for detecting recognition of a
25 peptide by T-cells comprises an antibody to a cytokine.

19. A kit according to claim 18, wherein said antibody is immobilised on a solid support and wherein said kit optionally comprises a means to detect an antibody/cytokine complex.

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20. A kit according to claim 18 or 19, wherein said cytokine is IFN- γ .

21. A method of ascertaining the stage of a *Mycobacterium tuberculosis* infection in a human comprising determining whether there is a differential T cell response to different antigens in the human.

5 22. A method according to claim 21 wherein T cell responses to one or more of Rv3879c, ESAT-6, CFP10, Rv3873, Rv3878, Rv1989c are measured.

23. A method according to claim 21 or 22 which is carried out to

- 10 (i) to determine whether the infection is recent or longstanding, or
(ii) to determine whether the human is latently infected or has disease, or
(iii) to monitor the effect of treatment.